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SHORT COMMUNICATION

A report on the association of influenza B virus with respiratory tract infection of hospitalized children in Saudi Arabia

Fahad N. Almajhdi *

Department of Botany and Microbiology, College of Sciences, and Center of Excellence in Biotechnology Research, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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Abstract Influenza viruses are recognized as one of the major causes of respiratory tract infection in young children and elderly people throughout the world. In this report, influenza B virus was detected when 200 clinical samples of nasopharyngeal aspirates, collected from hospitalized children aged from one month to three years with respiratory tract infections, were tested by Reverse Transcription Polymerase Chain Reaction (RT-PCR). PCR products on the conserved regions sequence of the non-structural gene identified the presence of influenza B virus in the clinical samples. This finding is important and it may become a real threat if not well considered. Due to massive mutations that influenza virus type B undergone, high protective measured should be taken. Therefore, further characterization and isolation of the virus are essentially needed.

1. Introduction

Influenza viruses are single-negative stranded RNA belong to the family *Orthomyxoviridae* (Palese and Young, 1982). Excess morbidity and mortality rate are associated with influenza viruses' infection especially during the winter season. Hemagglutinin (HA) and neuraminidase (NA) major variable surface glycoproteins of the virus and contribute essentially for virus entry and release from the host-cells during virus infection (Chi et al., 2005). HA and NA affected by antigenic changes (drift and shift) and recombination leading to recurrent outbreaks of influenza viruses. However, influenza B viruses use other mechanisms, such as insertion/deletion and reassortment with antigenically and genetically distinct co-circulating viruses, to generate genetic diversity (Xu et al., 2001). Influenza B has two distinct lineages that circulate, known as: B/Victoria/12/87 lineage and B/Yamagata/16/88 lineage. These two distinct lineages have co-circulated for about 10–12 years or more, with one or the other lineage usually predominating in various parts of the world (Tsai et al., 2006). Even though, these two lineages were originated separately, these two lineages were disseminated world wide (Shaw et al., 2002). In Saudi Arabia during Hajj, million of hajjis from across the globe intermingle with each other, creating an opportunity

* Tel.: +966 4677778; fax: +966 4675833
E-mail address: majhdi@ksu.edu.sa

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for transmission of any such new strain of influenza, and later spread it to all parts of the world within a short time. Four years back, Influenza Surveillance System was initiated in Saudi Arabia in Makkah region during Hajj 1426 Hijria, and has been gradually expended (Saudi Epidemiology Bulletin, 2006).

In fact, the range of illnesses associated with influenza viruses in children, and antigenic characteristics and similarity between vaccine antigens and influenza strains associated with infections in children attending King Faisal Specialist Hospital and Research Centre Riyadh was determined (Al-hajjar et al., 1999). There are two major reasons accounted for influenza viruses to be diagnosed and monitor carefully continuously every year. First, circulating strains of the viruses undergo constant antigenic drift in the HA and NA genes resulting in isolate variation affecting the efficacy of the vaccine application. Second, it also and more importantly may generate new assorted strain from major antigenic shift which could lead to pandemic world wide. Therefore, it is important to develop rapid, specific and validate diagnostic method in children hospitalized with respiratory illness not only for better therapeutic management, but also for improved detection and identification of the infection agents. In this report, influenza B virus from clinical samples was detected successfully.

2. Materials and methods

Approximately 200 nasopharyngeal aspirates in phosphate buffered saline were collected by the nurse staff of King Khalid University Hospital, Riyadh from children aged one month–three years, hospitalized with respiratory tract infection. Total RNA was extracted from patient samples (300 μL) using a commercial reagent QIAamp viral RNA mini kit and was subjected to RT-PCR. The cycling conditions for the PCR were: an initial activation step at 95 °C for 15 min; followed by 35 cycles at 94 °C for 30 s, 50 °C for 90 s and 72 °C for 90 s; and a final extension at 72 °C for 10 min, and a final incubation at 4 °C. One set of primer designed from non-structural protein (NS) gene was used for detection of influenza B virus (primer 1,5′-ATG GCC ATC GGA TCC TCA AC-3′; primer 2,5′-TGT CAG CTA TTA TGG AGC TG-3′). Our designed primers and PCR conditions were optimized and validated against known primers used routinely in our lab such as EMCV virus (data not shown). The PCR products were analyzed on 2% agarose gel and corresponding bands were detected and compared with marker size of 100 bp DNA ladder. The DNA bands of influenza B virus were visualized using Transillumination with a UV light source.

3. Results and discussion

Epidemics caused by influenza viruses have been a major public health challenge. Although not responsible for pandemics, influenza B virus infections contribute substantially to the disease burden each year and account for as much as half of all infections in some seasons (Zou et al., 1997). Recently, nucleic acids (NA) testing include RT-PCR is more reliable, specific and sensitive techniques used today replacing conventional culture technology. In this context, it is accurate and rapid for viral identification used in diagnostic laboratories. Consequently, it enhances medical management of influenza infection during outbreaks and improves and implements proper

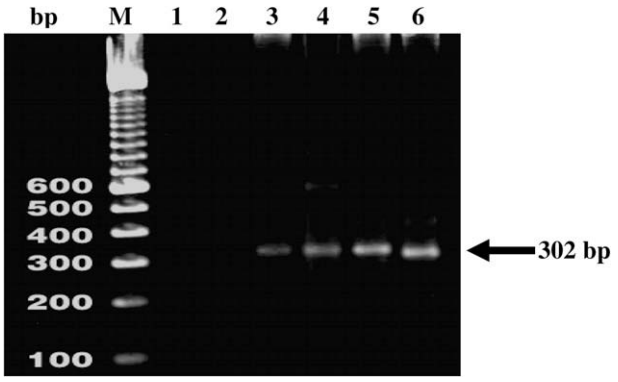

Figure 1 Detection of Influenza B virus using specific target sequence for non-structural protein (NS) gene from clinical samples. The annealing temperature for the PCR was set at 50 °C. M: 100 bp DNA Ladder Markers; Lanes 1 and 2: Influenza B negative, Lanes 3–6: Influenza B positive detected from clinical samples using specific primers designed from NS gene (302 bp).

antiviral therapy and treatment (Nicholson et al., 2003). In this study, it was demonstrated that RT-PCR method capable to detect influenza B virus from clinical samples using our specific primer designed from non-structural protein (NSP) gene. The Fig. 1 shows that the amplification of influenza viruses by RT-PCR detected influenza B virus (lanes 3–6). The size of amplification products of influenza B was 302 base pairs (bp). This size obtained in this study was correlated with those evaluated from NCBI Blast from primers information.

The virus was reported before in Saudi Arabia, but this is the first report describing influenza B detection from clinical samples directly using RT-PCR method in Saudi Arabia. This result is very important for influenza vaccine design based on the local circulating strains in Saudi Arabia as recommended by World Health Organization (WHO). Influenza B viruses have been circulating only in human despite the fact that the reservoir for influenza A viruses are human and other mammalian species (Wang et al., 2008). The latent ascribe the rationale accounts for several pandemics recorded for influenza A viruses. HA and NA genes of both type A and B influenza viruses are critical factors during the course of viral infection. HA and NA genes of the virus undergoes single or multiple point mutations leading to antigenic drift. In the other hand antigenic shift which induced by major genetic changes resulting from HA and NA genes substitutions in two different subtypes, is very rare in influenza B viruses but common in influenza A viruses (Lin et al., 2004; Xu et al., 2004). These changes on the antigenic sites (immune recognition sites) of the virus diminish the immune response against the virus infection and demonstrating the important of frequent monitoring and vaccine development each year for these new variants.

In this investigation, however, identification of the type B of the influenza virus spreading in Saudi Arabia between year 2005 and 2007 was reported and the work is now in process to further characterize the virus in order to fully understand the ancestry of the strain.

4. Conclusion

It was believed in this report that young children, who admitted to the hospital and suffer from respiratory tract infection,

are infected by influenza virus. According to the preliminary result we have, influenza B could be associated with theses complication which plays important role in the respiratory tract infection. Isolation and identification of influenza B virus are in process to further confirm of this finding.

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